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Publication date:
2012

Intestinal colonization, gut function and inflammatory responses are moderately influenced by gestational age at birth

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Suboptimal intestinal colonization is observed for many infant diseases, but it remains unknown if the divergent microbiota is mainly a result of disease or inborn characteristics such as maturational stage at birth, immune development and gut function. Preterm birth alters the gut microbiota which is considered a risk factor in necrotizing enterocolitis (NEC), a severe intestinal disease of preterm infants. However, it is unclear if gestational age or rather hygiene practices and antibiotic use determine the colonization differences between term and preterm newborns.

We hypothesized that gestational age at birth determines early gut colonization and immune development and that pigs born at different gestational ages but raised in the same environment differ in gut colonization, immune development and gut function.

Pigs were delivered by cesarean section at day 100 (n=4), 105 (n=18), 110 (n=14), and 115 (considered term, n=21) of gestation. Except for d100 pigs, all fed cow's colostrum, the groups were divided into diet groups and fed either formula or cow's colostrum via oro-gastric catheters. On day 3, all pigs were euthanized for tissue collection.

Small intestinal microbiology was assessed by 454-sequencing of bacterial 16S rDNA purified from luminal contents and subsequent analysis in the CLC Bio software. Totally 38 different bacteria were detected and of these only 11 were dominating (relative abundance >0.01). Gestational age minimally affected gut colonization but colostrum-feeding tended to increase *Clostridium perfringens* and *Streptococcus sp.* but decrease *Lactobacillus sp* across gestational ages.

Bacterial abundance in nine sections along the small intestine was assessed by fluorescence in situ hybridization using a 16S rRNA probe targeting the Bacterial Domain. Across sections and irrespective of diet, bacterial abundance was significantly reduced in d115-pigs compared to the preterm groups ($P<0.05$).

For all age groups, bacterial abundance nearly doubled in the mid and distal sections compared to the proximal.

Small intestinal expression of genes related to immune function and gut maturation was assessed by high throughput qPCR using a 48.48 Dynamic Array (Fluidigm) for 2304 simultaneous reactions. We used replicates of 26 primer pairs inclusive reference genes and found up-regulation (fold change >2) of genes related to pathogen sensing and inflammation (Il-1β, Il-8, NfκB and TLR2), barrier and gut function (MUC2, iAP, CLDN3, occluding and SGLT1) in d115-pigs and in some cases d110-pigs compared to pigs born more preterm. Across all ages, up-regulation of Il-8, TLR-4, TLR-2, C3 and MUC2 and down-regulation of iAP and lactase was observed after formula-feeding compared to colostrum-feeding. The dietary influence on gene regulation was most pronounced in the d105- and d110-pigs while it was almost absent in d115 pigs.

Although gestational age only affected gut colonization in regards to bacterial load this may affect sensitivity towards diseases such as NEC. The altered gut immune function possibly reinforces this sensitivity also through effects on the following colonization processes. However since diet affected gut colonization, immune development and gut function, dietary interventions may be used as a tool to counter act possible adverse effects of preterm birth.